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# **On the biology, ecology and early stages of *Rhagades (Wiegelia) predotae* (Naufock, 1930) (Lepidoptera: Zygaenidae, Procridinae)**

K. A. Efetov & G. M. Tarmann

## **Abstract**

The full life-cycle of *Rhagades (Wiegelia) predotae* (Naufock, 1930) is described for the first time. The DNA-barcode of this species is provided. *Prunus ramburii* Boiss. as the larval host-plant is mentioned.

KEY WORDS: Lepidoptera, Zygaenidae, Procridinae, *Rhagades predotae*, early stages, biology, DNA-barcode, Spain.

**Sobre la biología, ecología y primeros estadios de *Rhagades (Wiegelia) predotae* (Naufock, 1930)  
(Lepidoptera: Zygaenidae, Procridinae)**

## **Resumen**

Se describe, por primera vez, el ciclo de vida completo de *Rhagades (Wiegelia) predotae* (Naufock, 1930). Se proporciona el AND-código de barras de esta especie. Se menciona la planta nutricia *Prunus ramburii* Boiss.

PALABRAS CLAVE: Lepidoptera, Zygaenidae, Procridinae, *Rhagades predotae*, primeros estadios, biología, ADN código de barras, España.

## **Introduction**

The lepidopterous family Zygaenidae is represented by five subfamilies and more than 1000 species distributed in all zoogeographical regions (EFETOV, 1997a, 1997b, 1998a, 1999, 2000, 2001a, 2001b, 2006, 2010, 2018; EFETOV *et al.*, 2014a; EFETOV & TARMANN, 1999, 2013a, 2016b, 2017a, 2017b; MOLLET & TARMANN, 2018; TARMANN, 1994; TARMANN & COCK, 2019; TARMANN & DROUET, 2015). A lot of information has been obtained on this family during the last decades (EFETOV 1996a, 1998b, 1998c; EFETOV *et al.*, 2004, 2011b, 2015a, 2019b; EFETOV & KNYAZEV, 2014; EFETOV & SAVCHUK, 2009, 2013; KNYAZEV *et al.*, 2015; NAZARI *et al.*, 2019; NAZAROV & EFETOV, 1993). However, even in the Western Europe some species of Zygaenidae are still poorly investigated. For example, *Rhagades predotae* (Naufock, 1930), an endemic species of Spain (Fig. 12), was hitherto only known from a few specimens (DUTREIX & ESSAYAN, 1991; FERNÁNDEZ-RUBIO, 1995, 2005; FERNÁNDEZ-RUBIO & CUÑARRO-LARREA, 1996; VIVES MORENO & HUERTAS-DIONISIO, 1985). *Rh. predotae* belongs to the subgenus *Wiegelia* Efetov & Tarmann, 1995. The closest species is *Rh. (W.) amasina* (Herrich-Schäffer, 1851) (Figs 13, 15). One more *Rhagades* species is present in Spain, viz. *Rh. (Rhagades) pruni* ([Denis & Schiffermüller], 1775) (Figs 12-14).

There is only one publication with the description of the adult larva of *Rh. predotae* (VIVES MORENO & HUERTAS-DIONISIO, 1985). All other larval stages were unknown. To learn more

about its biology and ecology and to discover its full life-cycle and habits in nature G. M. Tarmann has already tried to find this species in July 1979 together with the late M. R. Gómez Bustillo in Noguera (province of Teruel) and Uña (province of Cuenca), but without success. In July 1980 during a second trip to the same localities three males were observed at Uña at sunset, but no females could be found. Then in June 2002 the authors of this paper made another attempt in Spain to obtain eggs or larvae of *Rh. predotae*. However, in this year only the larvae of *Aglaope infasta* L. were abundant on *Prunus* and we could not find any stages of *Rh. predotae*. Finally, success came in 2018. The basis of this result was the use of the new sex attractant created by the first author in the Crimean Federal University (EFETOV *et al.*, 2019a).

Sex pheromones and attractants are an efficient tool for studying Zygaenidae. In recent years application of the sex attractants has allowed us to gather rich information on the distribution and phenology of many species (CAN *et al.*, 2019; CAN-CENGIZ *et al.*, 2018; EFETOV *et al.*, 2010, 2011a, 2014b, 2015b; RAZOV *et al.*, 2017; SUBCHEV *et al.*, 2010, 2012, 2013, 2016; VRENOZI *et al.*, 2019). The first author with his team synthesized a series of new sex attractants 'EFETOV-2': enantiomers of 2-butyl 2-dodecanoate and their mixtures (EFETOV *et al.*, 2014c, 2016, 2018, 2019a). These attractants were applied by us during field work in 2018.

On 10 July 2018 we arrived at the locality Cuenca, 2 km NE of Huélar, at an altitude of 1222 meters. This is a place with flowery meadows surrounded by bushes of *Prunus*, *Crataegus* and *Rosa* (Fig. 1). At first we tried to move the branches of the *Prunus* bushes by shaking and beating them to disturb possible specimens of *Rhagades predotae* and force them to come out. But this was without success. However, at 20.38 hours, at the moment when the sun was descending behind the mountains, in the first evening twilight, one male came suddenly out of a bush of *Prunus* and approached the lure with the attractant EFETOV-S-S-2 (S-enantiomer) that was fixed on the hat of the second author. This was the only specimen seen that day. We spent the whole next day (11 July 2018) in the same place. We also set up three traps with sticky layers and attractants EFETOV-2 (racemic mixture of R- and S-enantiomers), EFETOV-S-S-2 and a control trap without attractant. On this day four male specimens of *Rh. predotae* were attracted to EFETOV-S-S-2 attached to the hats of the authors, one at 11.30 and three between 20.40 and 20.47 at the time of sunset. On the 12 July 2018 we checked the traps. All of them were empty. However, from 20.20 to 20.30, again at sunset, we once more observed four males attracted to the lures of EFETOV-S-S-2 on the hats of the authors. As we realised that with this method we only could obtain males and that no females were found during the whole day either on the wing or in the vegetation, we decided to look for another place. We arrived after sunset at a flat plain three kilometres to the North-East of Huélar, at an elevation of 1225 meters. As we could not see well enough in the darkness we turned the car around. However, suddenly, K. A. Efetov saw a specimen flying near the car. We went out and from 21.00 to 21.35 we found 18 males flying along the roadside in the upcoming darkness. The temperature at that time was still 23 degrees with a cloudless sky and no wind. In the morning of 13 July 2018 we returned to this place and found many small bushes of the Spanish endemic species *Prunus ramburii* Boiss. growing just along the roadside with their branches almost creeping along the ground (Fig. 2). We carefully checked the leaves of these plants and found 16 egg batches (Fig. 3) of *Rh. predotae* with egg numbers ranging from 5 to 100 (mean number 30). We also found one female beside an egg cluster. This specimen did not lay any eggs during the next few days in captivity. At 12.00 we also found one male and we observed that both the female and the male dropped to the ground when disturbed. Now it was clear why shaking the bushes was never successful. At 20.00 we returned to the same place and set up three attractants: EFETOV-2, EFETOV-S-2 (R-enantiomer) and EFETOV-S-S-2 on stones about 10 cm above the soil at a distance from each other of ca 10 meters (EFETOV *et al.*, 2019a). On this day sunset was at 20.45 (the time when the sun disappeared behind the mountain). Between 20.44 and 21.07 four males of *Rh. predotae* came to the attractant EFETOV-S-S-2, showing sexual activity by running around the lure, trembling their wings, turning their abdomens with exposed genitalia. No specimens were attracted to EFETOV-2 and EFETOV-S-2. Thus it became clear that EFETOV-S-S-2 is the attractant of *Rh. predotae* resembling the natural female sex pheromone of this species. It is interesting to know that no specimens were found in the sticky traps including those with EFETOV-S-S-2.

Beginning from 22 July 2019 first instar larvae emerged from the eggs and this was the material on the basis of which the early stages of the species were studied. It should be noted that most of the eggs were parasitized by small hymenopterous egg parasites.

### Description of the early stages of *Rhagades predotae*

**Ovum:** Oval, length 0.7 mm, breadth 0.5 mm, height 0.4 mm, pale yellow. Egg batch (Fig. 3) consists of one layer, eggs touch one another. The number of eggs in the batches varies from 5 to 100 (mean number is 30). Duration of egg stage 13 days.

**Larva:** FIRST INSTAR. Length of body 1.1-1.3 mm, breadth 0.5 mm. Body at first uniform yellowish white; head capsule brown, first thoracic segment with oval brown sclerotized dorsal plate (in some specimens the plate is not sclerotized medially forming two lateral sclerotized parts), thoracic legs brown (but lighter than head capsule), anal comb brown with 11 setae. Three days later light brown mediodorsal, dorsolateral and lateral lines appear on the body. The setal formula of the first abdominal segment is: D: 5-7d, 1l; SD: 3d, 1l; L: 0d, 2l (the terminology of the setae follows EFETOV *et al.* (2006) and EFETOV & HAYASHI (2008); D = dorsal setae, SD = subdorsal setae, L = lateral setae, l = light, hair-like setae, d = dark, stronger sclerotized setae). The combination of a few dark and one light subdorsal setae is typical for the genus *Rhagades* Wallengren, 1863 (EFETOV, 2001c) and is considered to represent an autapomorphy of the genus (EFETOV, 2004).

The larvae were reared on the leaves of *Prunus cerasifera* Ehrh. The first instar larva does not leaf-mine. It feeds on the parenchyma, at first forming a small round pit, later, by moving forward, forming an irregular short groove. Most of the first instar larvae prepare for moulting sitting on the surface of the leaves after they have spun a silky 'carpet' to fix their prolegs; only one larva moved into a fold of a leaf and additionally to the 'carpet' spun a few silk threads over the fold. Duration of L1: 5-6 days.

SECOND INSTAR. Length of body 1.5-1.6 mm. Body yellowish white with brown mediodorsal, dorsolateral, lateral and basal lines; head capsule, dorsal part of the first thoracic segment, thoracic legs and anal comb brown. Number of the setae increased, the setal formula of the first abdominal segment is: D: 10-11d, 2l; SD: 7d, 3l; L: 0d, 2l. Among the three light subdorsal setae one is longer than the two others; moreover, the light setae are situated more laterally than the dark ones.

The larvae feed on the parenchyma of the leaves but leave the epidermis from one side untouched and do not create holes; the feeding marks look like white spots (Fig. 4). Some of the second instar larvae produce small cocoons inside which they moult. Duration of L2: 4-5 days.

THIRD INSTAR. Length of body 2.5-3.0 mm. Body of the same colour as in the second instar, but the lines became darker. Number of the setae increased, the formula of the dorsal setae of the first abdominal segment is approximately 23d, 4l; among the four light dorsal setae two are longer than the two others.

The larvae feed on the parenchyma of the leaves but also a few holes appeared. Half of the third instar larvae moult in light cocoons. Duration of L3: 5 days.

FOURTH INSTAR. Length of body 3.5-4.0 mm. The colour of the body and verrucae became whitish grey; the lines are brown. Number of the setae increased, the formula of the dorsal setae of the first abdominal segment is approximately 25d, 6-7l; among light dorsal setae two are longer than others.

The larvae feed on the parenchyma and also create holes in the leaves. Half of the fourth instar larvae moult in light cocoons. Duration of L4: 6-7 days.

FIFTH INSTAR. Length of body 4.2-4.8 mm. The colour of the body whitish grey, but verrucae became yellowish grey, ventral surface and prolegs light yellow; the lines are brown (as in L3 and L4); head capsule, dorsal plate of the first thoracic segment, thoracic legs and anal comb dark brown (as in L3-L4). Number of setae increased, the formula of the dorsal setae of the first abdominal segment is approximately 27-28d, 7-9l; among light dorsal setae two are longer than others.

The larvae feed on the parenchyma and also create holes in the leaves (the total areas of the white

spots and the holes on the leaves are nearly equal). Half of the fifth instar larvae moult in cocoons. Duration of L5: 6-8 days.

SIXTH INSTAR. Length of body 4.5 mm (not longer than fifth instar). The colour of the verrucae whitish brown, ventral surface and prolegs light brown; the lines became darker, dark brown; head capsule, dorsal plate of the first thoracic segment, thoracic legs and anal comb dark brown. The formula of the dorsal setae of the first abdominal segment is approximately  $30d, 5-6l$ ; among light dorsal setae two are longer than others.

Between the end of August and beginning of October most of the larvae were sitting inside cocoons, then they came out and continued to feed, but not much (only few small feeding marks appeared). Duration of L6: 54-56 days (end of August - mid of October).

SEVENTH INSTAR. Length of body 4.0 mm (less than sixth instar). The larvae become darker (than the sixth instar), colour of verrucae brown, ventral surface and prolegs orange brown; the lines blackish brown; head capsule, dorsal plate of the first thoracic segment, thoracic legs and anal comb dark brown. Number of light setae on the dorsal verruca of the first abdominal segment is 8, all other setae are short and dark.

At first the larvae are still feeding but not too much, then they stop and most of them spend the winter in their cocoons (hibernacula). The larvae start feeding again in the beginning of April producing holes in the leaves and also start to feed at the margins of the leaves. Preparing for moulting the larvae sit in groups together inside a common silk network construction (like a "blanket"). Duration of L7: 6 months with hibernation (mid of October - mid of April).

EIGHTH INSTAR. Length of body 6.0-6.5 mm. Verrucae brown; cervical part of first thoracic segment, ventral surface of body and prolegs orange; lines blackish brown; head capsule, dorsal plates of the first thoracic and last abdominal segments, thoracic legs and anal comb blackish brown. Now there are three types of setae on the dorsal verrucae, viz. short brown with darker apices and two types of long white setae: simple and plumose, the latter with short lateral bristles extending from the central stem (see EFETOV, 2001c, fig. 94); these white plumose setae are situated on the anterior, lateral and posterior parts of the verruca (forming a lateral semicircle). Moreover, there are four types of setae on the subdorsal verrucae, in addition to the three types of setae mentioned before there are also light orange plumose setae forming two groups that are situated on the dorsoanterior and dorsoposterior parts of the verruca (on the first abdominal segment subdorsal verruca has respectively 4-5 anterior and 1-3 posterior orange setae). Thus, the plumose setae (white and orange) appear in eighth instar for the first time.

Larvae actively feed. Moult takes place under silk "blanket". Duration of L8: 15 days.

NINTH INSTAR. Length of body 7.5-8.0 mm. Verrucae dark greyish brown; cervical part of first thoracic segment, ventral surface of body and prolegs orange; mediobursal and dorsolateral lines blackish brown; head capsule, dorsal plates of the first thoracic and last abdominal segments, thoracic legs and anal comb blackish brown. There are three types of setae on the dorsal verrucae. First type: short, spine-like, brown, not plumose, with darker apices; these setae are situated on the central and medial parts of the verruca. Second type: long, hair-like, white, not plumose; these setae are situated on the anterior and posterior parts of the verruca. Third type: long, hair-like, plumose, white; these setae are situated on the anterior, lateral and posterior parts of the verruca. There are four types of setae on the subdorsal verrucae. First type: short, spine-like, brown, not plumose, with darker apices; these setae are situated on the central part of the verruca. Second type: long, hair-like, white, not plumose; these setae are situated on the inferior part of the verruca. Third type: long, hair-like, orange, plumose; these setae are situated on the dorsoanterior and dorsoposterior parts of the verruca, their number increased comparing with eighth instar (on the first abdominal segment subdorsal verruca has 8-12 such setae). Fourth type: long, hair-like, white, plumose. Lateral and subventral verrucae have only two types of setae: short, spine-like, brown, not plumose, with darker apices and long, hair-like, white, not plumose.

Larvae actively feed. Before moulting some larvae gather together in a corner of the rearing box

producing over themselves a common, not dense, silk network construction, like a “blanket” (Fig. 5). Duration of L9: 9-12 days.

TENTH INSTAR (FULLY GROWN LARVA) (Fig. 6). Length of body 11.0-11.5 mm. Colour of the body and setae as in ninth instar. Each of dorsal and subdorsal verrucae with one of the other long, hair-like, white, not plumose setae extremely long (4-5 times longer than the short, spine-like, brown setae). Number of orange plumose setae of subdorsal verrucae increased (16-19 on the verruca of the first abdominal segment). Second and seventh abdominal segments with greyish white evert lateral bulbs (“glands”) situated between subdorsal and lateral verrucae under the spiracle like in some other species of the Zyginaeidae (EFETOV & TARMANN, 2004). Dorsal sclerotized plate of the last abdominal segment arch-shaped. Anal comb narrow with high base and 9-12 processes.

Larvae feed on the leaves of *Prunus cerasifera* Ehrh. eating the whole leaf except central vein. Duration of L10: 18 days.

Cocoon: Length 12.5-13.5 mm. Spindle-shaped, white, constructed of loosely spun silk, semitransparent with a few white particles on the surface.

Pupa (Fig. 7): Length 8.3-8.5 mm. Head, thorax, wings and abdomen smooth, shiny; head, thorax and wings brown, abdomen yellowish light brown; proboscis well developed, its tip reaches distal margin of forewing and not extending beyond end of abdomen. Dorsally last abdominal segment and distal margin of penultimate segment with short light brown setae, their length 0.2 mm. Duration of pupal stage: 11-13 days. The imagines emerge during the morning.

Adults (Figs 8-10): Moths with yellow well developed proboscis which they use for feeding (Fig. 11). When the females attract the males, the posture of the female (Fig. 10) is such that the dorsal parts of the abdominal tergites are exposed between the wings. Before copulation the male shows sexual behavior with the antennae moving up and down, the wings trembling, and the abdomen bending with opened valvae.

Male genitalia with long uncus: half length of the aedeagus (in *Rhagades amasina* the uncus is short: 4-5 times shorter than aedeagus). The vesica with only one cornutus (in *Rh. amasina* with three cornuti) (EFETOV, 2001c, plate 1, fig. 2; plate 2, fig. 3).

Female genitalia with characteristic structure named “praebursa” which is present in many species of Procridiniae (ALBERTI, 1954; EFETOV, 1996b; EFETOV & TARMANN, 2013b, 2014a, 2014b, 2016a) and is the strongly dilated distal part of the ductus bursae between the antrum and the corpus bursae. In *Rh. predotae* the praebursa has no spines (in *Rh. amasina* it has a series of heavily sclerotized spines) (EFETOV, 2001c, plate 27, figs 2, 3).

## DNA barcode

DNA barcode (COI gene sequence) (EFETOV *et al.*, 2019c) of the male specimen of *Rh. predotae* (Spain, Cuenca, vic. Uña, 1300 m, 15-16-VII-1980, G. M. Tarmann leg.) is listed below.

Genbank number HQ987489:

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*****TAAGTCTATTAAATTCGAGCAGAATT
AGGTACTCCAGGATCTCTAATTGGAGATGATCAAATTATAATACTATTGTAACAGCTCATGC
TTTATTATAATTTTTTATAGTAATACCTATTATAATTGGAGGATTGAAATTGATTAGTAC
CCTTAATATTAGGAGCTCTGATATAGCCTTCCTCGTATAATAATATAAGATTGACTATT
CCCCCATCATTAATTCTTTAATTCAAGAAGAATTGTAGAAAATGGAGCAGGAACTGGATG
AACTGTATATCCCCCTTCTCCAATTGCTCACAGAGGAAGATCTGTTGATTAGTAAT
TTTTCTCTCATTAGCTGGAATCTCTCAATTAGGTGCAATTAATTCTACTACTATT
TTAATATACGACCTAATGGTATATTGATCAAATGCCATTATCGTATGAGCAGTAGGAAT
TACTGCTTTTATTACTTCACTCCAGTATTAGCAGGAGCAATTACTATACTTTAAC
GACCGAAATATTAATACTTCTTTTGATCCAGCAGGGGGAGGAGACCCATTCTTATCAA
CATTATT
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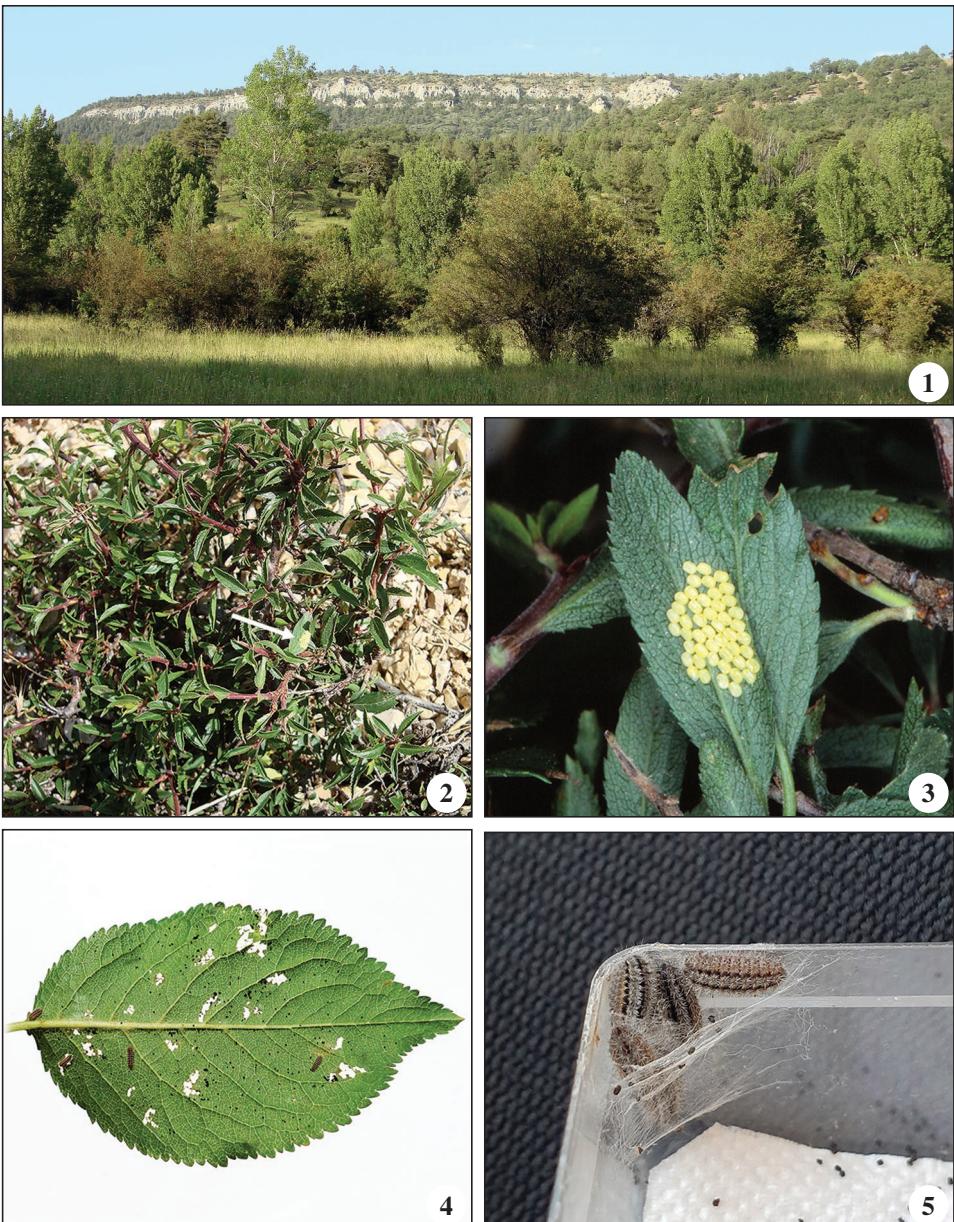
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**Figures 1-5.-** Biology of *Rhagades predotae*. **1.** Habitat of *Rh. predotae* at Spain, Castilla-La Mancha, Cuenca Province, NE of Huélar, 1222 m. **2.** *Prunus ramburii* Boiss., the larval host-plant. Eggs are shown by white arrow. **3.** Eggs on a leaf of the host-plant. **4.** Second instar larvae and feeding marks on the leaf of *Prunus cerasifera* Ehrh. (in captivity). **5.** Four ninth instar larvae preparing for moulting inside a common silk network construction.



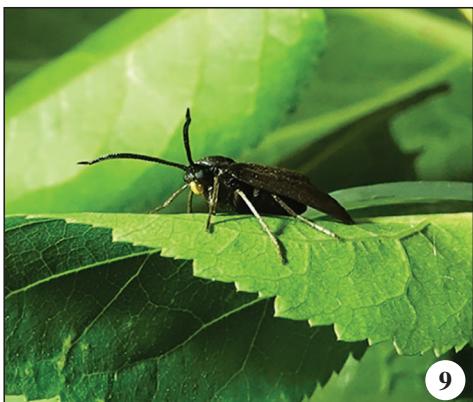
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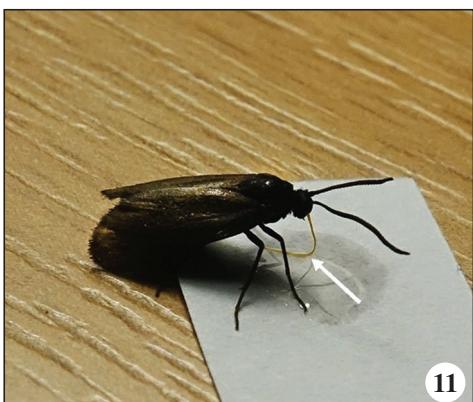
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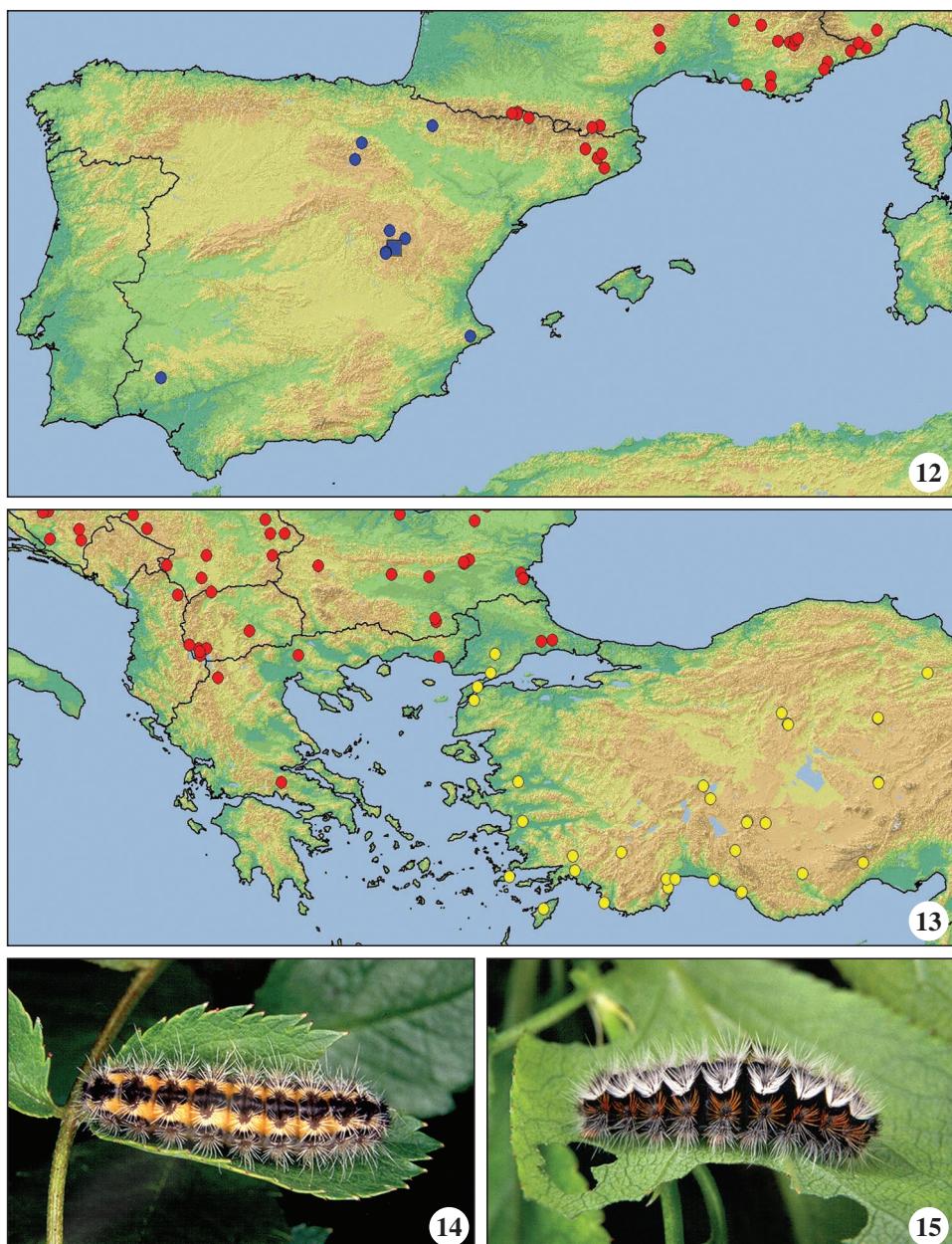


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**Figures 6-11.-** Biology of *Rhagades predotae*. **6.** Fully grown (tenth instar) larva. **7.** Pupa (ventral view). **8.** Freshly emerged male, reared from egg (Spain, Cuenca Province, NE of Hué lámo). **9.** Freshly emerged female, reared from egg (Spain, Cuenca Province, NE of Hué lámo), yellow proboscis is visible. **10.** Virgin female in calling position waiting for male. **11.** Female feeding from a solution of sucrose in captivity, the well-developed functionally active yellow proboscis is visible.



**Figures 12-15.**— 12. Distribution of *Rhagades predotae* (blue) and *Rhagades pruni* (red) on Iberian Peninsula (square shows locality vic. Huélamo). 13. Distribution of *Rhagades pruni* (red) and *Rhagades amasina* (yellow) on Southern Balkans, Aegean Islands and Western Anatolia. 14. Fully grown larva of *Rh. pruni* (after EFETOV, 2001c). 15. Fully grown larva of *Rh. amasina* (after EFETOV, 2001c).